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1616

Patent
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(RR-473PCT/US)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:

Chris C. Miller

Serial No.: 09/762,152

Filed: February 1, 2001

For: METHOD AND APPARATUS FOR TREATMENT OF RESPIRATORY INFECTIONS BY NITRIC OXIDE INHALATION

Group Art Unit: 1616

Examiner: Haghighatian, Mina

SUPPLEMENTAL COMMUNICATION

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Sir:

Applicant hereby submits the enclosed Certified Copy of Canadian Patent Application No. 2,254,645, filed November 23, 1998, from which the present application claim priority.

The Commissioner is authorized to charge Sidley Austin Brown and Wood's Deposit Account # **50-1597** for any fees necessitated by this filing, including the fee for any extension of time.

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If the undersigned can be of any assistance, please feel free to call the undersigned.

Respectfully Submitted

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This is to certify that the documents attached hereto and identified below are true copies of the documents on file in the Patent Office.

Specification and Drawings, as originally filed, with Application for Patent Serial No: **2,254,645**, on November 23, 1998, by **PULMONOX MEDICAL CORPORATION**, assignee of Chris Miller, for "Method and Apparatus for Treatment of Respiratory Infections by Nitric Oxide Inhalation".

Agent certificateur/Certifying Officer

December 10, 2003
Date

Canada

(CIPO 68)
04-09-02

OPIC CIPO

METHOD AND APPARATUS FOR TREATMENT OF RESPIRATORY INFECTIONS BY NITRIC OXIDE INHALATION

Field of the Invention

The present invention in one aspect relates to the use of nitric oxide gas (NO) in the treatment of fungal, parasitic and bacterial infections, particularly pulmonary infection by *mycobacterium tuberculosis*. In a second aspect, the invention relates to improved apparatus for the pulsed-dose delivery of nitric oxide for the treatment of microbial based diseases which we have found to be susceptible to nitric oxide gas. The device is designed to provide high dose nitric oxide replacement therapy for infected respiratory tract infections, or as a sterilizing agent for medical equipment.

Background of the Invention

In healthy humans, endogenously synthesized NO is thought to exert an important mycobacteriocidal or inhibitory action in addition to a vasodilatory action.

There have been a number of ongoing, controlled studies to ascertain the benefits, safety and efficacy of inhaled nitric oxide as a pulmonary vasodilator. Inhaled nitric oxide has been successfully utilized in the treatment of various pulmonary diseases such as persistent pulmonary hypertension in newborns and adult respiratory distress syndrome.

There has been no attempt, however, to reproduce the microbacteriocidal or inhibitory action of NO with exogenous NO. Our studies on the exposure on extra cellular *M. tuberculosis* to low concentrations of NO for short periods have led us to conclude that exogenous NO

exerts a powerful dose- and time-dependent mycobacteriocidal action from this and from promising *in vivo* studies, we have inferred that the large population of extracellular bacilli in patients with cavitary pulmonary tuberculosis are also vulnerable to exogenous (inhaled) NO.

Summary of the Invention

In one aspect the present invention is the novel use of inhaled nitric oxide gas as an agent for killing bacterial cells, parasites and fungi in the treatment of respiratory infections.

According to the present invention, there is also provided a portable battery-operated, self-contained medical device that generates its own nitric oxide as a primary source, and may also include a conventional compressed gas source of NO as a secondary back-up system. The device of the invention operates to deliver NO in the gaseous phase to spontaneously breathing or to ventilated individual patients having microbial infections, by way of a specially designed nasal-cannula or mask having a modified Fruman valve.

In a preferred embodiment of the invention, nitric oxide gas is produced in cartridges through thermal-chemical, ultrasonic and/or electrochemical reaction and is released upon user inspiratory demand in pulsed-dose or continuous flow.

Brief Description of the Drawings

The nature and scope of the invention will be elaborated in the detailed description which follows, in connection with the enclosed drawing figures, in which:

Figure 1 illustrates an airtight chamber for exposure of mycobacteria to varying concentrations of NO in tests of *in vitro* measurements of the cidal effects of exogenous NO;

Figure 2 is a graphical representation of experimental data showing the relationship of percent kill of microbes to exposure time for fixed doses of NO;

Figure 3A shows the external features of a pulse-dose delivery device for nitric oxide according to the present invention;

Figure 3B illustrates schematically the internal working components of the device of Figure 3A;

Figure 4 is a schematic illustration of the specialized valve used to control the delivery of nitric oxide in a preset dosage through the disposable nasal cannula of a device according to the present invention; and

Figure 5 is a schematic drawing of the mask-valve of arrangement of a pulsed-dose nitric oxide delivery device according to the present invention.

DETAILED DESCRIPTION OF THE INVENTION

Measurements of Cidial Activity of Exogenous NO

To re-create a normal incubation environment that allowed for the exposure of mycobacteria to varying concentrations of NO we built an airtight "exposure chamber" that could be seated in a heated biological safety cabinet (Fig. 1). This chamber measured 31 x 31 x 21 cm and is made of plexiglass. It has a lid which can

be firmly sealed, single entry and exit ports through which continuous, low-flow, 5-10% CO_2 in air can pass, and a thermometer. A "Y" connector in the inflow tubing allows delivery of NO, at predetermined concentrations, to the exposure chamber. Between the "Y" connector and the exposure chamber is a baffle box which mixes the gases. Finally between the baffle box and the exposure chamber is placed an in-line NO analyzer (Pulmonox® Sensor, Pulmonox Medical Corporation, Tofield, AB, Canada). This analyzer continuously measures NO concentration in the gas mixture entering the exposure chamber.

The day before an experiment a precise quantity of actively growing virulent *M. tuberculosis* was plated on solid media (Middlebrook 7H-10 with OADC enrichment) after careful dilution using McFarland nephelometry (1 in 10 dilution, diluted further to an estimated 10^3 bacteria/ml and using a 0.1 ml inoculate of this suspension)(11). Control and test plates were prepared for each experiment. Control plates were placed in a CO_2 incubator (Forma Scientific, Marietta, Ohio) and incubated in standard fashion at 37°C in 5-10% CO_2 in air. Test plates were placed in the exposure chamber for a pre-determined period of time after which they were removed and placed in the incubator along with the control plates. The temperature of the exposure chamber was maintained at 32-34°C. Colony counts were measured on control and test plates at 2, 3 and 6 weeks from the day of plating. Reported counts are those measured at three weeks expressed as a percentage of control.

Experiments were of two varieties: 1.) Those that involved exposure of the drug susceptible laboratory strain H37RV to fixed concentrations of NO, ie. 0 (sham), 25, 50, 70 and 90 PPM for periods of 3, 6, 12, and 24 hours, and 2.) Those that involved exposure of a

multidrug-resistant (isoniazid and rifampin) wild strain of *M. tuberculosis* to fixed concentrations of NO, ie. 70 and 90 PPM for periods of 3, 6, 12 and 24 hours. One experiment at 90 PPM NO, that used both strains of *M. tuberculosis*, was extended to allow for a total exposure time of 48 hours.

The NO analyzer was calibrated at least every third experiment with oxygen (0 PPM of NO) and NO at 83 PPM.

Statistical Analysis

For each No exposure time and No concentration studied at least two and in most cases three or four separate experiments were performed with 3-6 exposure plates per set. Colony counts performed on each exposure plate were expressed as a percentage of the mean colony count of the matched non-exposed control plates.

The values from all experiments at each NO concentration and exposure time were then averaged. These data were analyzed using two-way analysis of variance using the F statistic to test for independent effects of NO exposure time and NO concentration and of any interaction between them on the colony counts.

Experimental Results

A diagram of the incubation environment is shown in Figure 1. With two exceptions this environment exactly simulated the usual incubation environment of *M. tuberculosis* in the laboratory - first the temperature of our exposure chamber was maintained at 32-34°C rather than the usual 37°C to avoid desiccation of the nutrient media upon which the bacteria were plated, and second, the test plates were openly exposed. That a stable and comparable incubation environment was reproduced was

verified in four sham experiments using the H37RV laboratory strain of *M. tuberculosis*. Colony counts on plates exposed to 5-10% CO_2 in air (O PPM NO) at 32-34°C in the exposure chamber, were not significantly different from those on control plates placed in the laboratory CO_2 incubator at 37°, as in Table 1, below:

TABLE 1
COLONY COUNTS AFTER EXPOSURE OF THE LABORATORY STRAIN (H37RV) OF *M. TUBERCULOSIS* TO VARYING CONCENTRATIONS OF NITRIC OXIDE FOR PERIODS OF 3, 6, 12 AND 24 HOURS

Colony Counts (Mean ± SE) (expressed as percentage of control)				
	Exposure Time (Hours)			
NO (PPM)	3	6	12	24
0	107 ± 5 (6)*	100 ± 5 (6)	97 ± 9 (6)	105 ± 5 (18)
25	109 ± 6 (12)	109 ± 4 (12)	102 ± 3 (12)	66 ± 4 (18)
50	97 ± 5 (12)	96 ±2 (12)	69 ± 3 (12)	41 ±5 (18)
70	80 ± 10 (7)	63 ± 12 (7)	58 ± 12 (11)	21 ± 6 (11)
90	101 ± 15 (11)	67 ± 7 (11)	64 ± 7 (14)	15 ±3 (15)

* Numbers in brackets refer to the number of plates prepared for each NO concentration at each time interval.

Seventeen experiments of the first variety, where plates inoculated with a 0.1 ml suspension of 10^3 bacteria/ml of the H37RV strain of *M. tuberculosis* were exposed to a fixed concentration (either 0, 25, 50, 70 or 90 PPM) of NO for increasing periods of time (3,6,12 and 24 hours) were performed. The results have been pooled and are outlined in Table 1. There were both dose and time dependent cidal effects of NO that were very significant by two-way ANOVA (F ratio 13.4, $P < 0.001$; F ratio 98.1, $P < 0.0001$ respectively) and there was also a statistically significant interactive effect on microbial killing efficacy (F ratio 2.03, $P < 0.048$). Although

there was some variability in the percentage killed from experiment to experiment, increasing the standard error of the pooled data, the dose and time effect were highly reproducible. Only one control and one test (12 hour) plate at 90 PPM were contaminated. That the effect of NO was cidal and not inhibitory was confirmed by the absence of new colony formation beyond three weeks.

As described in Fig. 2, the response to a fixed dose of NO was relatively linear with the slope of the line relating exposure time to percent kill increasing proportionally with the dose. Dose-related microbial killing did not appear to increase above 70 PPM NO, since colony counts at 70 and 90 PPM were indistinguishable. At 24 hours of NO exposure at both the 70 and 90 PPM NO levels, more than one third of the exposed plates were sterile. One experiment at 90 PPM NO was extended to allow for a total exposure time of 48 hours; all of these plates were sterile (Fig. 2 and Table 2).

TABLE 2
COLONY COUNTS AFTER EXPOSURE OF A MULTIDRUG-RESISTANT WILD STRAIN OF *M. TUBERCULOSIS* TO NITRIC OXIDE FOR PERIODS OF 3, 6, 12, 24 AND 48 HOURS

Colony Counts (Mean ± SE) (expressed as percentage of control)					
	Exposure Time (Hours)				
NO (PPM)	3	6	12	24	48
70	113 ± 2(4)	75 ± 4(4)	85 ± 10(4)	66 ± 4(4)	
			50 ± 25(4)	10 ± 5(4)	
90	97 ± 11(2)	91 ± 11(2)	71 ± 8(2)	36 ± 10(2)	
			59 ± 4(4)	32 ± 3(4)	0 ± 0(4)
			79 ± 5(4)†	20 ± 3(4)†	0 ± 0(4)†

* Each series represents an individual experiment; numbers in brackets refer to the number of plates prepared for each experiment at each time interval.

† These results refer to the H37RV laboratory strain.

Four experiments of the second variety, where plates inoculated with a 0.1 ml suspension of 10^3 bacteria/ml of a multidrug=resistant wild strain of *M. tuberculosis*, were exposed to a fixed concentration (either 70 or 90 PPM) of NO for increasing periods of time (3, 6, 12 and 24 hours) were performed, two at each of 70 and 90 PPM NO. Again there was a significant dose and time dependent cidal effect (Table 2). Although the percent kill at 24 hours was less than that observed with the H37RV strain, when an inoculum of this strain was exposed to 90 PPM NO for a period of 48 hours there was also 100% kill.

Conclusion

Using an *in vitro* model in which the nitric oxide concentration of the incubation environment was varied we

have demonstrated that exogenous no delivered at concentrations of less than 100 PPM exerts a powerful dose and time dependent mycobacteriocidal action. When an inoculate of *M. tuberculosis* that yielded countable colonies (0.1 ml of a suspension of 10^3 bacteria/ml) was plated on nutrient rich media and exposed to exogenous NO at 25, 50, 70 and 90 PPM for 24 hours there was approximately 30, 60, 80 and 85% kill, respectively. Similarly when plates of the same inocula were exposed to a fixed concentration of exogenous NO, for example 70 PPM, for increasing durations of time, the percentage of kill was directly proportional to exposure time; approximately 20, 35, 40 and 80% kill at 3, 6, 12 and 24 hours, respectively. Of added interest, the dose and time dependent mycobacteriocidal effect of NO was similar for both the H37RV laboratory strain and a multidrug=resistant (isoniazid and rifampin) wild strain of *M. tuberculosis*, (after 24 and 48 hours exposure to 90 PPM NO, there was 85 and 100% kill and 66 and 100% kill of the two strains, respectively) expanding the potential therapeutic role of exogenous NO and suggesting that the mechanism of action of NO is independent of the pharmacologic action of these cidal drugs.

The dominant mechanism(s) whereby *intracellular* NO, known to be produced in response to stimulation of the calcium-independent inducible nitric oxide synthase, results in intracellular killing of mycobacteria is still unknown (5). Multiple molecular targets exist, including intracellular targets of peroxynitrite, the product of the reaction between NO and superoxide (12). Whatever the mechanism(s), there is evidence that NO may be active not just in murine but also in human alveolar macrophages, (6-9) and furthermore that this activity may be critical to the mycobacteriocidal action of activated macrophages. Whether macrophase inducible NOS produces NO that has *extracellular* activity is not known but it is

reasonable to expect that a measure of positive (mycobacteriocidal) and negative (tissue necrosis) activity might follow the death of the macrophase itself.

The relative ease with which NO may be delivered exogenously, and its theoretical ability to rapidly destroy the extracellular population of bacilli in the patient with sputum smear positive pulmonary tuberculosis, especially drug-resistant disease, have great clinical appeal.

Primary Unit of the NO Post-Delivery Device

Figure 3a

The main unit provides a small enclosure designed to hang on a belt. An internal rechargeable battery powers the unit if required. The user interface provides a multi-character display screen for easy input and readability (1). A front overlay with tactile electronic switches allows easy input from user to respond to software driven menu commands (2). LED and audible alarms provide notification to user of battery life and usage (3). A Leur-type lock connector (4) establishes communication with the delivery line to either the nasal cannula device or the inlet conduit on the modified Fruman valve (diagram 3-22). An A/C inlet provides an electrical port to provide power to recharge the internal battery (5).

Figure 4a

The main unit houses four main components. The first component or subassembly is the electronic/control portion of the device. It includes a microprocessor driven proportional valve, alarm system, electronic surveillance system and data input/output display system and electronic/software watch dog unit (1). The second

subassembly includes the disposable nitric oxide substrate cartridges and interface mechanism (3). The substrate converter system (3) processes the primary compounds and converts it into pure nitric oxide gas. The gas then flows into an accumulator stable (7) and is regulated by a proportional valve (5) into the outlet nipple (8). The third subassembly is the secondary or backup nitric oxide system. It consists of mini-cylinders of high nitric oxide concentration under low-pressure. This system is activated if and when the primary nitric oxide source is found faulty, depleted or not available (4).

Nasal Cannula Adjunt - Figure 5

This diagram is a detailed drawing of the valve used to control the delivery of Nitric Oxide in a preset dosage through a disposable nasal cannula shown. The valve is controlled by the natural action of spontaneous respiration by the patient and the dosage is preset by the physical configuration of the device.

This valve is constructed of dual lumen tubing (1). The internal diameter of the tubing depends on the required dosage. The tubing is constructed of material compatible with dry Nitric Oxide gas for the duration of the prescribed therapy. This tubing is glued into the nasal cannula port (2). (See the over all diagram of the entire nasal cannula.) The valve consists of a very flexible flapper (3) that is attached by a spot of adhesive (4) so as to be positioned over the supply tube. The flapper valve must be very flexible because the valve action is effected by the natural respiration of the patient. When the patient breathes in the lower pressure in the nasal cannula causes the flapper valve to open and the dry gas is delivered from the reservoir (10) past the valve (3) and into the patient's respiratory tract. When the patient exhales positive pressure in the nasal

cannula forces the flapper valve closed preventing any delivered gas entering the respiratory tract. The supplied gas is delivered at a constant rate through supply tube (5). The rate must be above that required to deliver the necessary concentration to the patient by filling the supply reservoir up to the exhaust port (8) during expiration. When the patient is exhaling the flapper valve (3) is closed and the supply gas feeds from the supply line (5) through the cross port (6) into the storage chamber (10). The length of the storage chamber (10) given as dimension (9) determines the volume of gas delivered when the patient inhales.

Inhaling opens the flapper valve and causes the supply chamber (10) to be emptied. During exhalation when the valve (3) is closed and the supply chamber (10) is filling, any excess gas exhausts through exhaust port (8). During inhalation, when the supply chamber is emptied the supply chamber is displaced with atmospheric air through exhaust port (8). There will continue to be supply gas from supply line (5) through the cross port during inhalation and this amount must be figured into the total delivered gas to determine the actual dosage.

The tubing lumens are plugged (7) to direct the flow.

Mask/Valve Adjunct - Figure 6

The nitric oxide valve utilized is a modification and improvement of a Non-rebreathing valve for gas administration US patent No. 3,036, 584. It has been specifically redesigned for use in inhaled nitric oxide therapy.

The valve body (3) has a mask or mouth-piece attached to it. The connection will be standarized to a 22mm O.D. to facilitate this. The other end of the valve

body chamber is the exhaust port (4). The exhaust port entrains ambient air during the latter portion of inpiration and dilutes the nitric oxide coming from the inlet conduit (5). The resultant nitric oxide concentration in the valve body (3) is determined by the dilutional factors regulated by the valve, tidal volume and the nitric oxide concentration in the flexed bag. The inlet conduit (5) will be spliced and a small flexed bag (1) will be attached. The purpose of the bag is to act as a reservoir for nitric oxide gas. The opening on the inlet conduit (2) will be modified to facilitate the supply hose that emanates from the nitric oxide supply chamber.

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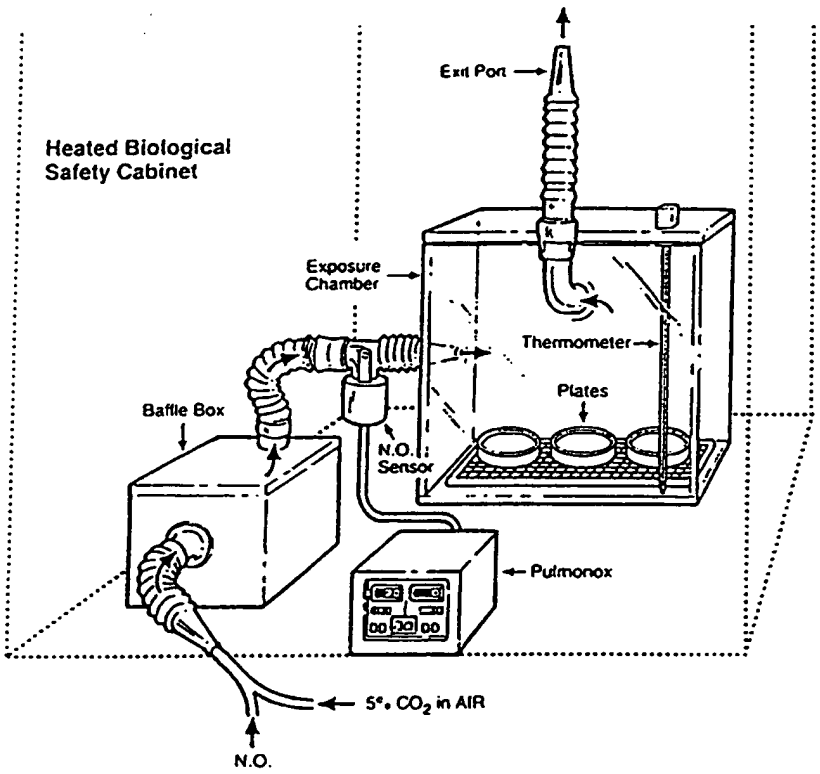

Heated Biological
Safety Cabinet
Exit Port
Exposure
Chamber
Thermometer
Plates
Baffle Box
N.O.
Sensor
Pulmonox
5% CO_2 in AIR
N.O.

FIGURE 1

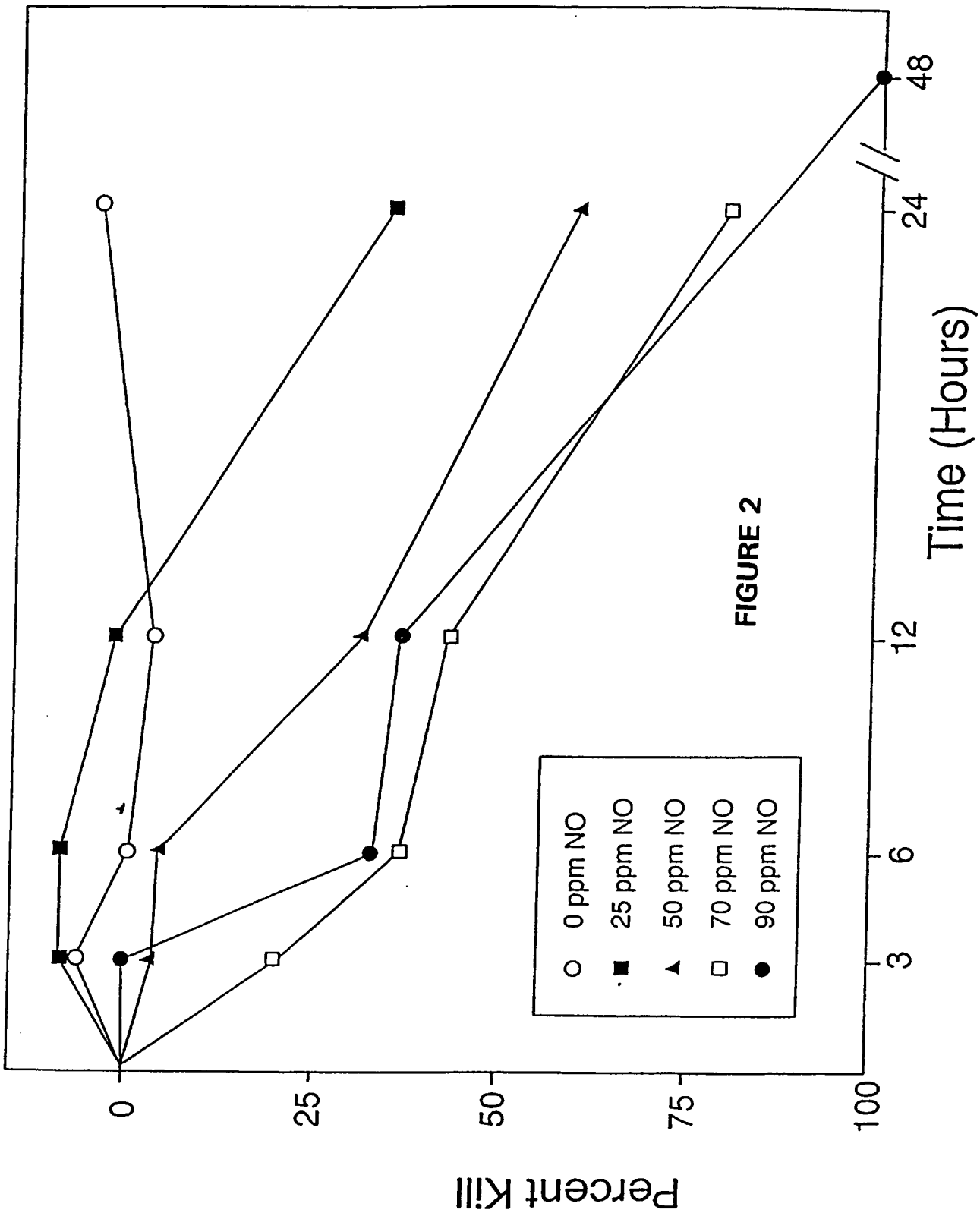

Percent Kill
0
25
50
75
100
3
6
12
24
48
Time (Hours)
0 ppm NO
25 ppm NO
50 ppm NO
70 ppm NO
90 ppm NO

FIGURE 2

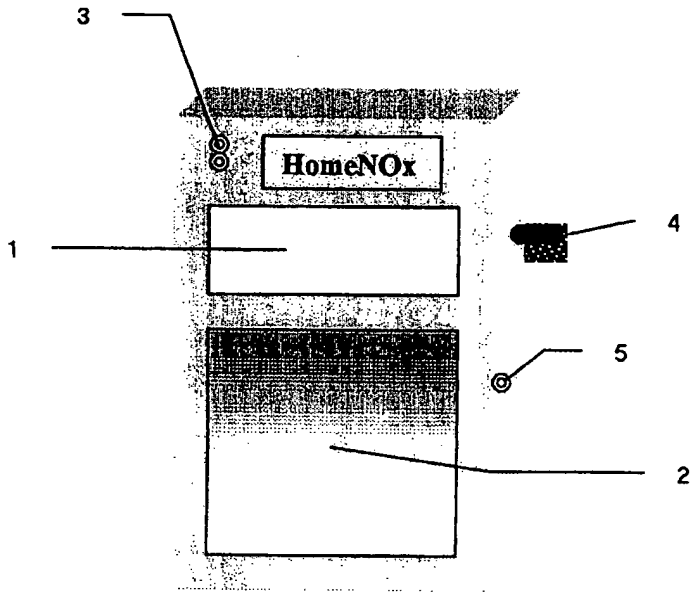


FIGURE 3A

1 – Display Screen
2 – Front Overlay & Membrane Switches
3 – Main/Battery Power LED
4 – Delivery Outlet
5 – A/C Input

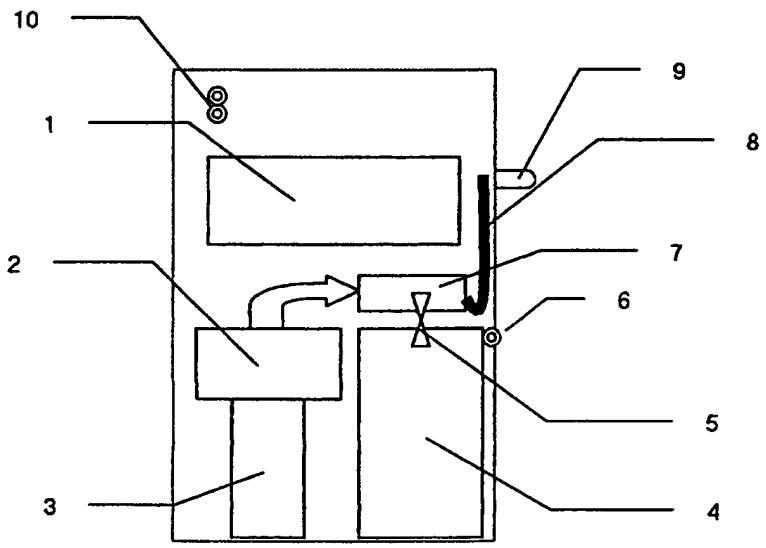
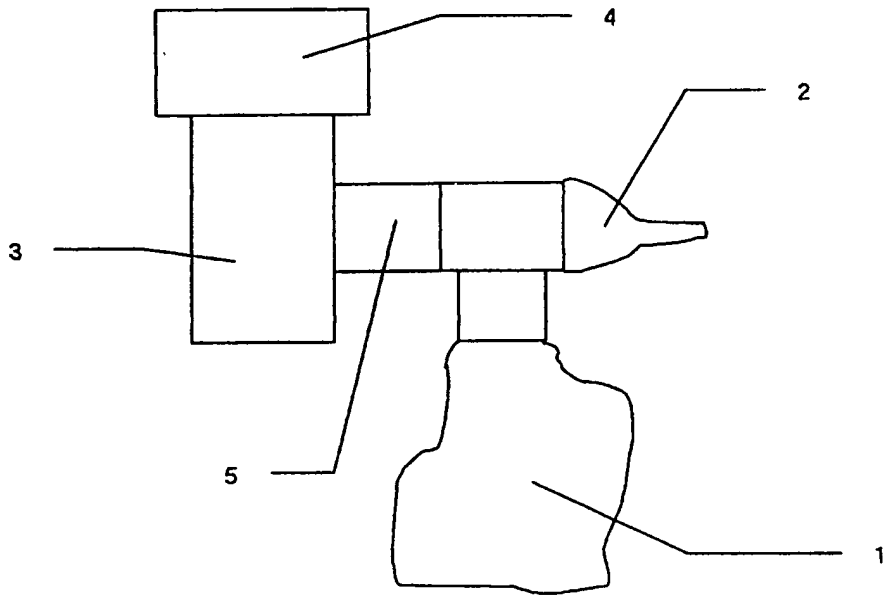


FIGURE 3B

1 – Display Screen
2 – Substrate Converter Segment
3 – Disposable Nitric Oxide Substrate Cartridge
4 – Secondary Nitric oxide Source (Back-up)
5 – Proportional Valve Assembly
6 – A/C Inlet (Rechargeable Battery)
7 – Accumulator Stable
8 – Nitric Oxide Outlet Nipple
10 –Main/Battery LEDs



1 – Flexed reservoir bag
2 – Knurled hose barb connector
3 – Valve body (ref 10 in patent diagram)
4 – Exhaust port (ref 11 in patent diagram)
5 – Inlet conduit (ref 22 in patent diagram)

FIGURE 4

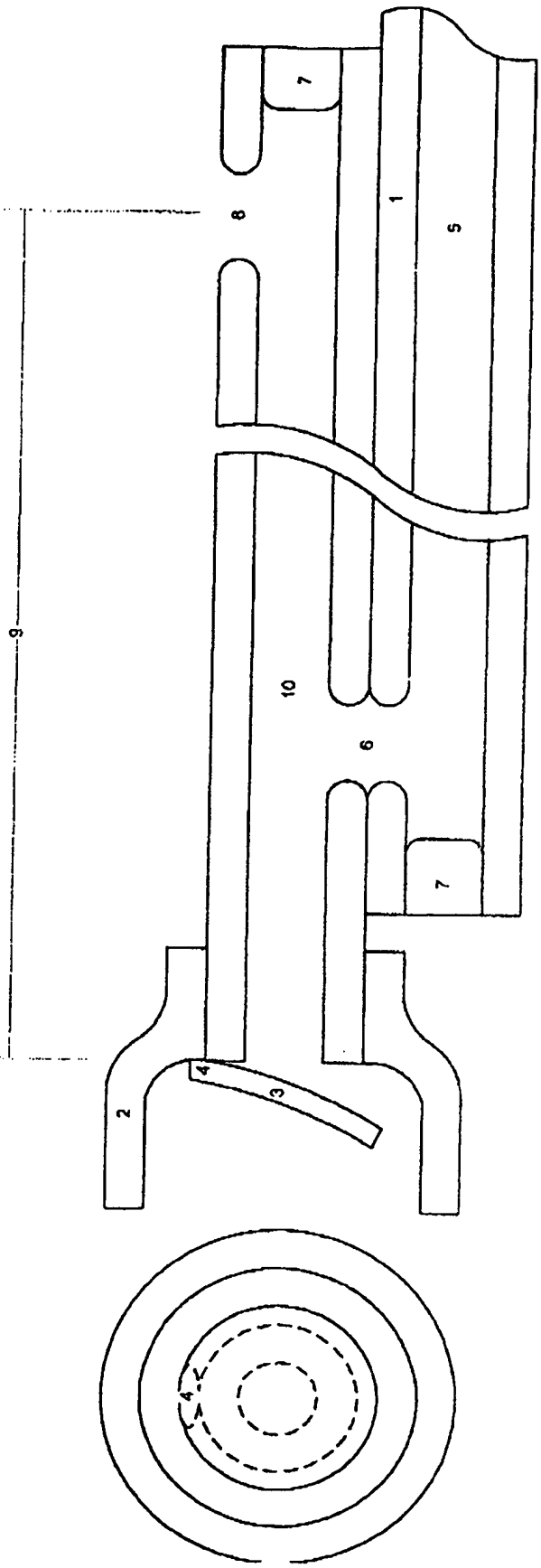


FIGURE 5